Replication in perivascular meningeal macrophages precedes meningitis in mice

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Background

Streptococcus pneumoniae is the leading cause of bacterial meningitis in children younger than 5 years of age in the US, and is the leading cause of death due to communicable disease (CDC, 2020; Wang et al., 2016). Following the onset of bacteremia, haematogenous spread of pneumococci across the blood brain barrier (BBB) results in the invasion of the CSF and meninges, which precedes the secretion of pro-inflammatory cytokines and neutrophil influx resulting in the onset of meningitis and brain injury (McNeela et al., 2010; Hassané et al., 2017). Pneumococci are known to adhere to endothelial cells prior to translocation across the endothelial barrier, but knowledge on the interaction of pneumococci with macrophages and neutrophils following this translocation event is scarce (Iovino et al., 2017; Uchiyama et al., 2009). To study these interactions, we utilised an in vivo murine model in which cefazolin was administered 12 hours after the induction of bacteremia to prevent septicaemia without penetrating the BBB.

Pneumococci replicate within perivascular macrophages following translocation of the endothelium

Cefazolin was administered at 12h post-infection (PI) following intravenous infection of mice with type 2 pneumococcal strain D39. Pneumococci were recovered from the brain by 24 h PI, and counts increased significantly up to 72h PI alongside consistently negative pneumococcal blood counts (Fig 1A). Confocal image analysis of brain samples revealed preferential localisation of single pneumococci with endothelial cell membranes within the choroid plexus (CP) and meninges at earlier time points (Fig 1B), before being associated primarily with perivascular (PV) macrophages at later time points, presenting as intracellular clusters (Fig 1B). During this intracellular period, neutrophils were absent from the CP and meninges and displayed no signs of meningitis inflammation. At 72h PI, pneumococci were predominantly extracellular, and an influx of neutrophils was visualised in close proximity to extracellular pneumococci (Fig 1F). These findings imply that following endothelial translocation by pneumococci, PV macrophages permit intracellular replication which allows bacterial numbers to increase before lysis and release into the CSF, therefore inducing neutrophil influx.

PECAM blockage, Caspase-1 inhibition and prevention of pneumococcal uptake into PV macrophages decreases brain bacterial counts

To investigate potential approaches that could lower pneumococcal brain counts, we administered an anti-PECAM antibody prior to infection and an anti-CD169 antibody after 8h infection (to negate effects on CD169+ macrophages in the spleen prior to brain invasion) to prevent pneumococcal endothelial adhesion and uptake into PV macrophages respectively. Pneumococci also induce NLRP3 inflammasome formation in brain phagocytes which, through the action of Caspase-1 and subsequent pyroptotic cell death, results in the release of pro-inflammatory cytokines (Wittenrath et al., 2011; Hoegen et al., 2011). Thus, we also administered VX765 Caspase-1 inhibitor prior to infection to prevent Caspase-1 activation in PV macrophages. Administration of anti-CD169 antibody significantly lowered the association of pneumococci with PV macrophages and significantly reduced brain pneumococcal counts at 48h PI (Fig 3AB). This implies that uptake into PV macrophages is essential for an increase of pneumococcal numbers in the brain. Anti-PECAM administration also resulted in ~62% of mice yielding no pneumococci in the brain, therefore demonstrating that a blockage of endothelial adhesion can successfully reduce the numbers of pneumococci crossing the BBB. Further, inhibition of Caspase-1 through administration of VX765 resulted in negative brain pneumococcal counts in ~87% of mice. The mechanisms responsible for this reduction in counts is unknown, however we hypothesise that prevention of the pyroptotic pathway could result in the cells resorting to the apototic pathway which successfully kills intracellular bacteria.

Human meningitis brain samples illustrate similar pneumococcal pathogenesis to mice

Brain samples from two patients who succumbed to pneumococcal meningitis were stained for IHC as above. Microscopy analysis revealed the presence of pneumococci associated with endothelial cells and in the CSF in the temporal cortex and hippocampus PV tissue. Large clusters of pneumococci were also found within PV macrophages in these regions (Fig 2AB), and neutrophils were found in sites of extracellular pneumococci accumulation, oftentimes in close proximity to macrophages that display morphological features in keeping with cell lysis (Fig 2C).

Summary

Following endothelial adhesion, pneumococci translocate the BBB, are phagocytosed by PV macrophages where they replicate, and are released following cell lysis resulting in neutrophil influx. Blockage of endothelial adhesion, uptake into PV macrophages and inhibition of pyroptosis can significantly lower brain pneumococcal counts.

References